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Editorial

Oral biosciences: The annual review 2015

A B S T R A C T

Background: The *Journal of Oral Biosciences* is devoted to the advancement and dissemination of fundamental knowledge concerning every aspect of oral biosciences.

Highlight: This review article features the following topics: "Novel challenge for bone formation and bone resorption," "The front line of research on oral microbiota," "Clinical insight into the study of orofacial pain," "Carving a disease by omics," "The front line of bioimaging—a new light shining on oral biosciences," "Biodental engineering—integration of biology and material science," "Translational dental research over the CCN family," "Salivary glands," "Break the negative spiral consisting of periodontitis, diabetes, and Alzheimer's disease: extending healthy life expectancy through oral health," "Immunology and oncology," "Oral microbiome and biofilm research: new concepts and new approaches," "Bone remodeling mechanisms of bone resorption and bone formation," and "The front line of oral biofilm research," in addition to review articles by invited authors in the field of microbiology.

Conclusion: These reviews published in the *Journal of Oral Biosciences* have inspired the readers of the journal to broaden their knowledge regarding various aspects of oral biosciences. The current editorial review introduces these exciting review articles.

1. Introduction

In addition to original articles, the *Journal of Oral Biosciences* also publishes review articles by the prizewinners of the "Lion Dental Research Award" and "Rising Members Award," presented by the Japanese Association for Oral Biology. The Journal also publishes review articles featuring recent information presented in symposia held during the annual meeting of the Association. In 2015, we published special issues featuring the following reviews: "Novel challenge for bone formation and bone resorption," "The front line of research on oral microbiota," "Clinical insight into the study of orofacial pain," "Carving a disease by omics," "The front line of bioimaging—new light shining on oral biosciences," "Biodental engineering—integration of biology and material science," "Translational dental research over the CCN family," "Salivary glands," "Break the negative spiral consisting of periodontitis, diabetes and Alzheimer's disease: extending healthy life expectancy through oral health," "Immunology and oncology," "Oral microbiome and biofilm research: new concepts and new approaches," "Bone remodeling mechanisms of bone resorption and bone formation," and "The front line of oral biofilm research," in addition to the review articles by the invited authors in the field of microbiology. These reviews published in the *Journal of Oral Biosciences* have inspired the readers of the journal to broaden their knowledge regarding various aspects of oral biosciences. The current editorial review introduces these exciting review articles.

2. Novel challenge for bone formation and bone resorption

It is well known that sympathetic nerve activity and hormone serum levels show circadian rhythms, and have been identified in most animals [1–3]. Although circadian rhythms are also prevalent in bone metabolism, the underlying molecular mechanisms are poorly understood. Recently, clock genes were discovered to be the modulators of circadian rhythmicity in animals. In their review article [4], Kondo and Togari focused on their recent findings regarding the circadian regulation of bone metabolism by β-adrenergic signaling, glucocorticoids, and clock genes [5–7]. They demonstrated that β-adrenergic signaling and/or glucocorticoids are mediators of circadian rhythms from the suprachiasmatic nuclei (SCN), where the main circadian rhythm is regulated by endogenous circadian clocks, to peripheral osteoblasts. In addition, glucocorticoids are one of the most important factors in the transmission of circadian timing from the SCN to peripheral osteoclasts. Finally, the osteoclast peripheral clock may regulate the circadian rhythm of bone resorption by regulating the expression of osteoclast-related genes such as cathepsin K (*mCtsk*) and nuclear factor of activated T-cells cytoplasmic 1 (*mNfatc1*) [6]. Thus, the clock gene, muscle Arnt-like protein (BMAL), contributes to osteoclast circadian rhythm.

Regarding osteoclast differentiation from hematopoietic stem cells, recent studies have found that two proteins, receptor activator of NF-κB (RANK) and its ligand (RANKL), are crucial for osteoclast development [8,9]. It has been reported that mice lacking both NF-κB1 and NF-κB2 develop typical osteopetrosis accompanied by a dramatic reduction in osteoclast number due to defective tracking of the osteoclast lineage [10,11], suggesting that osteoclast differentiation depends on RANKL-induced NF-κB activation in the osteoclast precursors. A recent study reported that in vitro inactivation of NF-κB using specific inhibitors and in vivo expression of dominant-negative IKKβ in osteoblasts enhanced osteoblastic bone formation [12], suggesting that NF-κB regulates both osteoblastic bone formation and osteoclastic bone resorption.

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In their review article [13], Jimi and colleagues focused on the recent discoveries, highlighting the roles of the "classical" and "alternative" NF-κB signaling pathways in bone morphogenetic protein (BMP) induced osteoblast differentiation and bone formation, and discussed the possibility that inhibition of NF-κB might promote BMP-induced bone regeneration for the treatment of bone diseases. Activation of the classical and alternative NF-κB pathways negatively regulates osteoblastic bone formation by modulating BMP/Smad signaling through two distinct mechanisms: inhibition of BMP-induced Smad DNA binding, and Smad1/5/8 phosphorylation [14–16]. Specific inhibitors of the NF-κB pathways seem to be efficient not only in preventing bone loss, but also in stimulating bone formation, which is referred to as "One bite provides dual tastes." Thus, NF-κB-selective inhibitors may have the potential to improve BMP-induced bone regeneration.

3. The front line of research on oral microbiota

Anaerobic cultural methods continue to expand our understanding of the oral microbiota of periodontitis and dental caries, although approaches for strain identification have evolved from biochemical tests to 16S rRNA sequence-based identification. Tanner, in her review article [17] based on her findings [18–22], focused on selective anaerobic culture studies that have provided the basis of our understanding of the oral microbiota. Non-cultural molecular analyses of plaque samples, mainly based on analysis of the 16S rRNA gene, have highlighted both the strengths and limitations of the culture-based methods in describing the complete oral microbiota. Nevertheless, when bacteria are detected by molecular methods, the focus then becomes to devise methods to cultivate them [23] which frequently involves the use of anaerobic methods. Anaerobic culture of bacteria associated with advanced periodontitis and dental caries, compared to healthy, non-diseased, sites has proven extremely valuable in expanding our knowledge of the bacteria associated with these major clinical conditions of the oral cavity. Anaerobic culture studies have enabled the rapid detection and identification of species using molecular methods that can be used in studies of larger populations of subjects. However, it can process only a limited number of samples. Furthermore, species in lower proportions of the overall microbiota, and species for which the nutritional requirements are as yet unknown, remain undetected by anaerobic culture [23]. A finding of clinical importance is that the pathogens in advanced periodontal and carious lesions were detected in the initial stages of the disease as well, suggesting that they can be suitable candidates for disease risk assessment. Since dental pathogens may also colonize healthy sites, assessment of periodontal and caries risk will require the addition of other risk markers—for example, host factors in periodontitis [24], and diet in dental caries [25].

Oral malodor in humans has long been a major health concern and may serve as a useful assessment tool for evaluating patients in a critical condition. Epidemiological studies have found that poor oral hygiene is associated with an increased risk of squamous cell carcinoma of the head, neck, and esophagus [26,27]; some have shown that the association might be causal [28–30]. Malodor originating in the oral cavity is an indicator of the metabolic output of the oral microbial communities as a whole. It is possible that the oral malodorous gases indicate not only halitosis, but also the pathogenicity of oral microbiota. In their review article [31], Tanda and colleagues focused on the role of microorganisms in producing malodorous gases, as well as the development of analytical techniques for the treatment of halitosis. Since most oral malodor originates from microbial activities in the mouth [30], and microbial activities cause aspiration pneumonia in hospitalized patients [32], oral malodor can serve as an indicator of the oral condition of critically ill patients. The oral cavity is easily exposed to tobacco smoke and alcohol, which are not only prominent risk factors for carcinogenesis, but also the strongest factors that increase microbial acetaldehyde production [33]. Hydrogen sulfide has been recognized as both a major cause of halitosis and as a gaseous-signaling molecule that might modulate cell physiology [34]. Continuous advances in the analytical techniques examining oral malodorous gases for the treatment of halitosis would enable better risk assessment of aspiration pneumonia and oral cancer in the future. Furthermore, metabolic approaches to oral malodor [35] may also elucidate the mechanisms underlying the production of gaseous metabolites relevant to these diseases.

4. Clinical insight into the study of orofacial pain

Allodynia and/or hyperalgesia frequently occur in the orofacial region following trigeminal nerve injury or orofacial inflammation [36]. Pathological pain associated with such an injury/inflammation is severe and difficult to treat, and occurs in wide areas innervated by the injured as well as uninjured nerve fibers. Similar symptoms have been observed in uninflamed as well as inflamed areas [37]. It is very important to understand the mechanisms underlying extraterritorial orofacial pain associated with trigeminal nerve injury or orofacial inflammation in order to develop appropriate measures for the treatment of patients with extraterritorial orofacial pain. Sugimoto and colleagues described recent findings in animal models, and the future directions of investigations of pathological pain mechanisms in their review article [38]. They summarized the current understanding of orofacial pain mechanisms as follows: (1) neurotransmitters are released from the somata of trigeminal ganglion (TG) neurons involved in peripheral sensitization; (2) the neurotransmitters released from the TG neurons are depressed after botulinum toxin-type A (BoNT/A) administration, suggesting that BoNT/A decreases neurotransmitter release to reduce neuropathic pain behavior; (3) glial cells are involved in the orofacial pathological pain associated with trigeminal nerve injury or orofacial inflammation along with the trigeminal spinal subnucleus caudalis and C1–C2 nociceptive neurons; (4) the trigeminal sensory nuclear complex, especially the trigeminal spinal subnucleus oralis, is structurally and functionally involved in orofacial pain sensations in normal and pathological pain conditions after peripheral nerve injuries; (5) neuroimaging analyses have suggested functional changes in the central and peripheral nervous systems in neuropathic pain conditions.

5. Carving a disease by omics

The CCN family is a group of matricellular proteins with six distinct members in mammals; of these, CCN2 is required for the proper development of the olfactory central nervous system [39], pancreas [40], hair follicles [41], and skeletal system [42] and is involved in multiple steps of orofacial development [43,44]. Specifically, CCN2 is important for skeletal development with the support of complex gene regulatory systems in the relevant cells, including osteoblasts, chondrocytes, and osteoclasts [42,45–47]. Kubota and colleagues focused on the biological roles of CCN2 in different microenvironments in their review article [48]. Since the proteins of the CCN family perform their functions through the manipulation of multiple molecular counterparts in the microenvironment, via molecular networks, the biological outcomes yielded by these proteins are sometimes unpredictable. Through combinatory investigation with metabolomic and transcriptomic

analyses, CCN2 was found to support various biological activities by enhancing the glycolytic production of ATP via the enolase 1 gene. Further trans-omic approaches such as interactomics are needed to identify and characterize more CCN2 molecular counterparts that would help obtain a comprehensive understanding of CCN2 functionality. Furthermore, this may lead to the discovery of novel functional aspects of this unique extracellular signaling modulator. The findings regarding the tissue regeneration potential and the critical role of CCN2 in fibrotic remodeling in a variety of tissues would be beneficial in establishing new CCN2-mediated therapeutics in clinical medicine and dentistry.

6. The front line of bioimaging—new light shining on oral biosciences

Fluorescent molecular imaging can be used to visualize biological processes in live cells, tissues, and animals. This method enables characterization of the localization and dynamics of particular molecules at the cellular and subcellular levels, and has become an indispensable technique for understanding many biological processes [49–51]. Molecular biology-based engineering of fluorescent proteins (FPs) has allowed researchers to design and create a variety of fluorescent indicators capable of detecting ions, intracellular messengers, phosphorylation, cell cycle stages, cell death, and other molecules or events [49–54]. Tanimura, in his review article [55] based on his findings [56,57], focused on FP-based fluorescent indicators that detect the concentration of intracellular messenger molecules. The development of sophisticated light microscopy techniques and fluorescent indicators has allowed for the visualization of dynamic processes in living cells. These imaging methods are particularly suitable for studying Ca^{2+} signaling, and have contributed to the discovery of spatially and temporally organized responses such as Ca^{2+} waves and oscillations [58]. FP-based indicators enable the monitoring of intracellular signaling, enzyme activities, apoptosis, cell cycle development, and other cellular processes. These indicators are delivered to the target cells or tissues as genetic material and are subsequently produced by the cells, either transiently or stably. Hence, they are suitable for long-term experiments, both in vitro and in vivo. This advantage of FP-based indicators extends their application and contributes to understanding the functions of particular molecules and cellular events during physiological responses in live animals.

Osteocytes are the most predominant cells in the bone and are located inside the mineralized bone matrix. Their cytoplasmic processes form a complex intercellular network via gap junctions, suggesting that osteocytes are the principal cells responsible for sensing mechanical stimuli and transporting signals that coordinate the adaptive bone-remodeling response [59]. Although mechanical loading-induced matrix strain is thought to cause a flow of interstitial fluid around the osteocyte processes [60,61], the mechanism by which this fluid flow excites the osteocytes remains unclear. Therefore, it is quite important to obtain precise morphological and/or morphometrical data from osteocytes and their surrounding matrix. In his review article [62] based on their findings [63–65], Kamioka and colleagues introduced the application of confocal laser scanning microscopy [66] to characterize the osteocyte network in the bone. They also described a high-resolution analysis of osteocyte morphology via ultra-high voltage electron microscopy (UHVEM) [67]. Finally, they demonstrated how the fluid flow causes fluid shear stress around the osteocyte processes using computer simulation based on a three-dimensional nanoscale model of osteocyte canaliculi. They visualized the entire osteocyte network in the bone as well as the microstructure of osteocyte cell processes and the surrounding bone matrix and found that the fluorescent analysis is useful for studying the osteocyte network morphology. Furthermore, using a three-dimensional image-based model of two distinct canaliculi, they found that the microscopic surface roughness of the canalicular wall strongly influenced the profile of the mechanical loading-induced flow of interstitial fluid, whereby highly inhomogeneous flow patterns emerged. These inhomogeneous flow patterns may induce the deformation of cytoskeletal elements in the osteocyte processes, thereby amplifying mechanical signals.

Tooth germs develop via epithelial–mesenchymal interactions, and the molecular mechanisms regulating this process have been elucidated in various studies. Several conserved signaling molecules have been implicated in the mediation of the epithelial–mesenchymal interactions that regulate morphogenesis and cell differentiation in tooth development. These molecules include fibroblast growth factors (FGFs), BMPs, Wnts, sonic hedgehog (Shh), and Notch [68]. There is a wealth of data on the expression patterns and functions of developmental regulatory molecules (See http://bite-it.helsinki.fi/). However, previous studies have not focused on direct observation of cell dynamics, and none has directly observed cell movement in the tooth germ. An organ culture system for tooth development had been designed, and two-photon laser microscopes have been used to observe cell division and cell fate. Although there is no universally accepted technique for observing cell fate at present, imaging has increased our understanding of tooth organogenesis and would contribute to the future elucidation of the mechanisms involved. Harada and colleagues discussed the benefits and limitations of live cell imaging with respect to previous studies in their review article [69]. Although time-lapse imaging is a strong tool for understanding cell movement and cell lineages, it is necessary to create a live imaging system for effective observation of cell dynamics to elucidate the mechanisms regulating tooth development.

The major salivary glands of humans and other mammals, including the parotid gland (PG), submandibular gland (SMG), and sublingual gland (SLG), are exocrine organs that secrete fluids, electrolytes, and proteins into the oral cavity. These glands are comprised predominantly of two epithelial cell types, acinar and ductal cells [70]. Salivary secretion is regulated primarily by the sympathetic and parasympathetic nervous systems [71]. Parasympathetic stimulation induces fluid and electrolyte secretion through the activation of muscarinic acetylcholine receptors (mAChRs) on salivary acinar cells. This activation is linked to the breakdown of phosphoinositide, which in turn induces inositol 1,4,5-trisphosphate (IP_3)-mediated Ca^{2+} release from intracellular Ca^{2+} stores, followed by Ca^{2+} influx across the plasma membrane. The mechanism of Ca^{2+} signaling in salivary gland cells was extensively examined using in vitro experiments employing organic fluorescent Ca^{2+} indicators, such as Fura-2 [72]. In particular, live cell Ca^{2+} imaging of salivary acinar cells revealed spatiotemporal changes in calcium ion concentration ($[Ca^{2+}]_i$), including Ca^{2+} waves and Ca^{2+} oscillations. Recent advances in imaging technologies, including fluorescent microscopy and the development of highly sensitive genetically encoded Ca^{2+} indicators (GECIs), have enabled the visualization of Ca^{2+} responses in live animals through intravital Ca^{2+} imaging in salivary acinar cells [73]. In their review article [74] based on their findings [75,76], Nezu and colleagues summarize in vitro studies of Ca^{2+} responses in salivary acinar cells. In addition, they described an intravital Ca^{2+} imaging method that enabled them to analyze the role of Ca^{2+} responses in salivary gland function in live animals. To examine the relationship between Ca^{2+} responses and salivary secretion in vivo, they developed an intravital imaging method to monitor Ca^{2+} responses in SMG acinar cells in vivo and successfully visualized agonist-induced increases in $[Ca^{2+}]_i$ in SMG acinar cells in live animals. This novel experimental system allowed them

to monitor Ca^{2+} mobilization during salivary secretion in live animals and could be a powerful tool for elucidating the relationship between Ca^{2+} responses and salivary secretion induced by various agonizts and the autonomic nervous system in vivo.

Fluorescence imaging represents a considerable breakthrough in biomedical science by facilitating new ways of observing molecular and cellular functions [77,78]. Although fluorescence imaging is widely used as a tool to confirm research hypotheses by high-resolution and colorful images, the technology is advantageous in that quantitative data can also be derived from the acquired images. Histological analysis has long been a powerful and informative approach, providing spatial information in tissues; however, such analyses often lack quantitative information. Fluorescence imaging with quantitative analysis is considered a suitable method for spatial and temporal data collection, as well as for functional quantification. In their review article [79] based on their findings [80,81], Lee and Iimura introduced the general methodology of quantitative fluorescence imaging and discussed its applications by reviewing their recent findings in osteocyte function. Quantitative fluorescence imaging is undoubtedly a powerful approach to uncover unprecedented biomedical phenomena. To exclude misinterpretation and subjective deductions from imaging data, multiple approaches and multimodality will be increasingly emphasized. Image processing and mathematical analyses would play an important role in providing further critical information, especially in vivo experiments.

7. Biodental engineering—integration of biology and material science

As a complimentary approach, biologists have begun to engineer biological materials themselves, including genes, molecules, cells, and tissues [82–85]. Induced pluripotent stem (iPS) cells may provide insights into the most basic questions within the field of cell differentiation, and into the capabilities of iPS cells in molecular and cellular biology [86,87]. iPS cells have also been reported to have potential clinical applications for the treatment of many diseases, particularly in the fields of regenerative medicine and tissue engineering [88–90]. Thus, the emergence of new research fields bridging engineering and biology is expected to provide a more comprehensive understanding of basic biology, as well as advancements in technological applications. In dentistry, the new field of biodental engineering integrates the sciences of dentistry and engineering. In their review article [91], Matsumoto and colleagues highlighted and discussed four examples of biodental engineering applications and advancements. Egusa and colleagues demonstrated that gingival fibroblasts (GFs), easily obtained from gingival tissues, can be readily reprogrammed into iPS cells without Myc transduction or a specific system for the selection of successfully reprogrammed cells, thus making them a promising source of cells for investigating the basis of cellular reprogramming and pluripotency for future clinical applications [92,93] to lead a variety of potential uses for both biological and treatment outputs. Kato and colleagues have focused on the specific expression patterns of cell surface markers to characterize stem cell populations. For this purpose, they have developed special material surfaces onto which multiple antibodies specific for surface markers were immobilized in an array format [94,95]. This can lead to the development of valuable tools for obtaining information on different aspects of stem cell populations. Tsuji and colleagues proposed a third technique, termed the three-dimensional organ-germ culture method. With this technique, they generated a tooth with the correct structure, using both in vitro organ culture and transplantation into an alveolar socket in vivo [96–98]. This study provides important insights into how cells self-assemble and function during tooth and salivary gland development. Matsumoto and colleagues also fabricated a large three-dimensional (3D) mesenchymal stem-cell (MSC) construct with a ball-like morphology (which they termed a cell ball), and cultured this 3D cell ball under hypoxic conditions, which causes chondrogenic differentiation of MSCs in primordial cartilage, a process that is crucial for ossification [99,100]. In the last half-century, biologists had begun to isolate themselves from researchers in other scientific fields. This so-called reductionist approach to biology is now being replaced with a more comprehensive, integrated approach, and researchers in biomedical fields are collaborating with other scientists in chemistry, engineering, materials science, and physics to develop new approaches to address old problems. Such is the case in the emerging field of biodental engineering, which combines biological sciences, engineering, and dentistry to develop methods, techniques, and technologies for both basic and applied sciences. Advancements in this field would provide new therapeutic approaches for improving the quality of life of dental patients.

8. Translational dental research over the CCN family

Matrix metalloproteases (MMPs) are generally thought to be involved in the degradation of extracellular matrix (ECM) components in the dental pulp. Interestingly, some MMPs, including MMP-2, MMP-3, MMP-9, and MMP-13, were also shown to be internalized by cells [101–104]. CCN family member 2/connective tissue growth factor (CCN2/CTGF) is a secretory protein belonging to the CCN family. CCN2/CTGF is a well-known osteogenesis- and chondrogenesis-related protein necessary for tooth development [105,106]. A previous report showed that CCN2/CTGF plays a role in the MMP-3-induced migration of human dental pulp cells, and the endocytosis-related protein dynamin mediates MMP-3-induced CCN2/CTGF expression [107]. Furthermore, bone morphogenetic protein (BMP)-1 participates in reparative dentinogenesis via CCN2/CTGF expression, and both MMP-3 and BMP-1 are shown to induce these effects independent of the protease activity. Therefore, the role of metalloproteases is not limited to catalytic enzyme activity during ECM remodeling; it also includes dental pulp repair. In the latter process, CCN2/CTGF is implicated as a key factor. In their review article [108], Muromachi and colleagues focused on novel roles of metalloproteases, specifically MMP-3 and BMP-1, and discuss the action of CCN2/CTGF in dentin–pulp complex repair. The classical role of metalloproteases as catalytic enzymes has recently been expanded to include their novel function as signaling molecules. However, it remains to be determined which cell surface molecules interact with MMP-3 and BMP-1. Further research on the relationship between metalloproteases and CCN2/CTGF expression would help shed light on their role in dentin–pulp complex regeneration and repair.

9. Salivary glands

Vacuolar H^+-ATPase (V-ATPase) is localized in the membranes of intracellular organelles such as vacuoles, lysosomes, the Golgi apparatus, and synaptic vesicles. Intracellular V-ATPase is important for membrane trafficking processes, including receptor-mediated endocytosis, protein processing and degradation, and the coupled transport of small molecules such as neurotransmitters [109]. V-ATPase is also expressed in plasma membranes, contributing to proton transport across the membrane of specialized cells, and acidifying adjacent lumina or interstitial spaces in some organs [110,111]. The functional role of V-ATPase remains unknown in the salivary gland [112]. The present review

by Sahara and colleagues overviewed V-ATPase in salivary glands, especially the cellular location of V-ATPase and its role in luminal acidification [113]. In addition, how acidification is regulated in the salivary glands was discussed by referring to the mechanism in the pancreatic duct, or collecting duct of the kidney. Advances in the phenotypic analysis of increasing numbers of mutants have helped gain an overview of the biological and physiological significance of individual subunits [114,115]. Although redundancies, compensatory mechanisms, and adaptive responses can occur, null mice of several subunit isoforms will be useful in better understanding their functions [116]. Studies defining the role of V-ATPase in the salivary ducts await confirmation of its presence at this site. Accumulating physiological knowledge, together with structural studies and progressive clinical experiments, could provide new insights and a significantly better understanding of the mechanism of action of V-ATPase and its vital contribution to life and health.

10. Break the negative spiral consisting of periodontitis, diabetes and Alzheimer's disease: extending healthy life expectancy through oral health

An estimated 36 million people worldwide have Alzheimer's disease (AD) or related dementias. The development of treatments that delay the onset of AD by five years is estimated to potentially reduce the number of AD patients by half. Recently, considerable attention has been focused on lifestyle related diseases, including diabetes [117] and periodontitis [118–121], as exacerbating factors for AD. Nakanishi and Wu focused on the strategies to prevent AD progression in their review article [122]. Low-grade chronic systemic inflammatory signals associated with these diseases may activate primed or senescent microglia, which subsequently provoke an exaggerated neuroinflammation. Furthermore, the bidirectional relationship between periodontitis and diabetes may amplify chronic systemic inflammatory signals that contribute to AD progression. Moreover, oral bacteria and their virulence factors infiltrate the brain parenchyma, provoking neuroinflammation. These observations indicate that periodontitis and diabetes promote AD progression. Furthermore, activation of the oral sensory system during mastication is a well-established contributor to brain health. Conversely, orofacial pain reduces the quality of life and contributes to both eating disorders and malnutrition in elderly people. Therefore, oral brain science strategies should be established for extending healthy life expectancy through brain health, which originates from oral health.

11. Immunology and oncology

During the past two decades, the notion that inflammatory events can promote cancer growth has experienced a vigorous renaissance, fueled by the development of new animal models of cancer, and rapid advances in the fields of immunology and inflammation [123–125]. These discoveries have led to a model positing that inflammation acts as a facilitator of cancer growth. In their review article [126], Postler and Ghosh focused on the molecules that have been linked to various cancers. Two classes of cellular regulators have emerged as particularly dominant players: the Ras family of small guanosine triphosphatases (GTPases), members of which are mutated in human solid cancers more frequently than any other type of gene, and the NF-κB family of transcription factors, which are the central regulators of inflammation and growth. Ras and NF-κB signaling are involved in such a breadth of biological processes that any broad-acting inhibition of either or both pathways is likely to have severe systemic side effects and thus be unsuitable as chronic treatment for long-term illnesses, such as cancer or chronic inflammation. Therefore, targeted intervention at the level of more specific modulators of signaling offers a more appealing strategy than shutting down the entire pathway. For instance, overexpression of κB-Ras proteins does not abrogate NF-κB signaling completely but modifies the expression of a subset of IκB-β-dependent target genes [127–129]. Similarly, overexpression of κB-Ras does not eliminate Ras signaling but acts specifically on the Ral pathway, which is essential for anchorage-independent proliferation, while leaving PI3K-dependent signaling intact [127,130–132]. Nonetheless, the expression of κB-Ras proteins is sufficient to significantly reduce the growth of tumor cells [127]. Thus, therapeutic intervention focusing on specific modulators of signaling may open up effective but safe new approaches to chemotherapy.

12. Oral microbiome and biofilm research: new concepts and new approaches

Xerostomia is a clinical condition caused by dry oral mucosa, or a reduction or absence of salivary flow. The estimated worldwide prevalence of xerostomia among elderly individuals (over 65 years old) is approximately 30% [133]. It has been reported that xerostomia is caused by radiotherapy for head and neck cancer, Sjögren's syndrome, poor glycemic control in diabetes, or obesity [134–137]. Thus, a precise understanding of the qualitative alterations of the oral microbiota caused by dry mouth, and appropriate oral care should help prevent caries and candidiasis; consequently, the prophylaxis of oral diseases would contribute to improve quality of life (QOL) in the elderly. In their review article [138] based on their findings [139], Hayashi and colleagues have discussed hyposalivation, clinical findings, and qualitative alterations of the oral microbiota, in order to prevent oral diseases caused by dry mouth. Using culturing of the microbiota and molecular biological assessment of 16S rDNA to evaluate the relationship between saliva secretion and the oral microbiota, they showed that hyposalivation contributes not only to fluctuations in the numbers of certain microorganisms, but also influences the composition of the microbiota (the microbial ecosystem) in the oral cavity. Furthermore, *Candida* sp., which prefers a dry environment, appears to increase and replace the normal flora under such conditions. Such alterations are reflected by clinical indices, such as the number of decayed teeth, decayed, missing, or filled Teeth (DMFT), and the prevalence rate of candidiasis in the clinical studies. Hyposalivation may not be directly related to the onset of periodontal disease. It is reasonable to assume that hyposalivation is an indirect risk factor for the progression of periodontal disease due to the higher supragingival plaque index associated with lower levels of saliva. In order to treat health concerns associated with hyposalivation (mastication disorder, anorexia, dysphagia, oral mucosal inflammation, risk of caries, and periodontal diseases), more attention should be paid to global alterations of the microbiota and prophylaxis of oral diseases.

13. Bone remodeling mechanisms of bone resorption and bone formation

BMPs induce ectopic bone formation when implanted into muscle tissue and stimulate osteoblast differentiation of various cell types [140,141]. BMP signaling is transduced by two types of transmembrane serine/threonine kinase receptors, namely, type I and II receptors [142,143]. SMAD signaling plays a central role among the downstream effectors of BMP receptors and plays an important role in osteoblast differentiation [144]. Both Osterix and

RUNX2 are essential factors for osteoblast differentiation, and their expression is activated by the BMP-SMAD signaling pathway [145,146]. BMP signaling and BMP-induced osteoblast differentiation are negatively regulated at each step by various factors. In their review article [147] based on their findings [148–150], Kokabu and colleagues focused on BMP signaling and its inhibitory mechanisms during osteoblast differentiation. In addition, they discussed strategies for treating bone-related diseases such as osteoporosis as well as for the tissue engineering of the bone by using BMPs. BMP signaling and its regulators were introduced, with a special emphasis on inhibitory factors. Reducing the activity or expression of these inhibitory factors could be useful for increasing endogenous BMP signaling. Developing methods of releasing these inhibitory mechanisms may shed light on novel bone formation therapies.

14. The front line of oral biofilm research

From birth, the infant is exposed to and colonized by a wide range of microorganisms, derived mainly from the mother. The biological properties of each habitat determine which microorganisms can colonize and grow, and determine the major and minor components of the resident microbiota of a site. This results in different surfaces having distinct but characteristic microbiotas [151–155]. In their natural environment, microorganisms revert to their so-called 'biofilm phenotype', and down-regulate certain activities and up-regulate the production of polymeric substances that act as viscoelastic inter-cellular binding material and extracellular energy storage compounds, amongst other roles [156,157]. This mode of existence offers protection against external stresses and promotes interactions among neighboring microbial cells [158], as well as between the biofilm and the host, resulting in a complex and dynamic interplay. In their review article [159] based on their findings [160–162], Marsh and colleagues focused on the oral microbiota that provides major benefits to the host including: (a) colonization resistance, (b) down-regulation of potentially damaging host inflammatory responses, and (c) active contributions to the normal development of the physiology of the mouth and the host defenses. The oral microbiota is natural and beneficial to the host, and disease is associated with shifts in the balance of the normal resident microbiota. In this way, dental diseases represent examples of (minor) ecological catastrophes, in which the oral microbiota is disrupted in response to a change in the local environment [163]. This has implications for treatment, and opens up potentially new avenues to control or prevent disease. Therefore, when a patient presents with a dental disease, a clinician should attempt to determine the factors responsible for driving dysbiosis, while recognizing that these could vary from patient to patient. These factors could include impaired saliva flow, poor oral hygiene techniques, inappropriate lifestyle, poor dietary habits, an impaired immune system, or the presence of other risk factors. Unless there is an attempt to interfere with or alter the factor(s) driving the dysbiosis, the patient is likely to keep returning to the surgery suffering from further episodes of the disease. An appreciation of the ecological principles may lead to opportunities in the future to manipulate the composition and metabolism of the oral microbiota in order to maintain the benefit we derive from their presence and activity, while minimizing the impact of any environmental and lifestyle factors that might lead to dysbiosis.

15. Microbiology

The bacterial etiology of chronic periodontitis is acknowledged to be polymicrobial in nature. There is a consensus that the anaerobic, proteolytic, amino acid-fermenting species *Porphyromonas gingivalis* plays a significant role in either initiation or progression of the disease [164–167]. Based on animal model data, *P. gingivalis* has recently been proposed to be a "keystone pathogen" that manipulates the host response to favor the proliferation of a pathogenic polymicrobial biofilm (dysbiosis) and the development of the disease [166]. A previous report demonstrated in a longitudinal human study that the imminent progression of chronic periodontitis could be predicted by increases in the relative levels of *P. gingivalis* and/or *Treponema denticola* in subgingival plaque [168], which is consistent with other clinical studies demonstrating that *P. gingivalis* levels in subgingival plaque are predictive of human disease progression [169–171]. *P. gingivalis* is also capable of causing periodontitis in animal models of the disease [172,173]. Reynolds and colleagues focused on the finding that *P. gingivalis* has novel heme, iron, and manganese transporters and metalloregulatory proteins that enable it to switch rapidly between an energy-efficient iron-dependent virulent phase and a protective manganese-dependent survival phase in their review article [174]. The clear interplay between iron, manganese, heme, and oxidative stress protection may enable the anaerobic *P. gingivalis* to maintain a high level of intracellular ferrous iron to maximize growth and virulence using energy-efficient iron-dependent metabolism, and to rapidly replace this potentially deadly metal with manganese for survival during oxidative stress by switching to a more protective, but much more restrictive, manganese-based physiology.

Conflict of interest

The author has no conflict of interest to declare.

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